Genetic background of early life telomere length and association with dairy cow longevity

Proceedings of the World Congress on Genetics Applied to Livestock Production, 11.498

Genetic background of early life telomere length and association with dairy cow longevity

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Summary

Involuntary culling is a major cause of economic loss in dairy cows. Here we examine the genetic background of telomere length (TL) and its association with dairy cow longevity, using the largest dataset of TL measurement in livestock species. Heritability of TL at birth (TLB) was moderate and significant. A genome-wide association study (GWAS) revealed three suggestive genome-wide significant SNPs on chromosomes 1 and 23 affecting TLB. Significant phenotypic correlations of TLB with a range of longevity traits and a significant genetic correlation with survival to 48 months of age were also estimated.

Keywords: longevity, dairy, telomere length, biomarker, GWAS

Introduction

Telomeres are tandem repeated DNA sequences capping chromosome ends. In vitro studies show that they are truncated during each mitotic cycle; once a critical length is reached, their protective function is lost and cells enter a stage of replicative senescence. The link between TL and cell age has thus led to a hypothesis that it could be used as a predictor of the organism's longevity; this was later confirmed in some studies on humans and wild animals (e.g. Cawthon et al. 2003), however the results have not been consistent.

In dairy cows, involuntary culling is a major source of economic loss, with genetic progress limited by low heritability and difficulties in measuring longevity. Therefore, a trait measured early in life, which could predict the lifespan of a cow could improve returns. TL is proving to be a likely candidate, with preliminary results showing its significant and positive associations with dairy cow longevity (Brown et al. 2012).

Estimates of TL heritability vary between studies and species, from 0.2 to 0.8 (e.g. Reichert et al. 2015). Recent GWAS studies in humans identified several single nucleotide polymorphisms (SNPs) and genes associated with TL in humans (e.g. Codd et al. 2013). However, the genetic background of TL in cattle remains largely unknown.

The objective of this study was to investigate the genetic architecture of leukocyte TL at birth using pedigree and genomic data, and test its associations with longevity in dairy

cattle.

Material and methods

Data

Data included 662 female Holstein Friesian cattle from the SRUC Dairy Research Centre at Crichton Royal Farm, Scotland. The Langhill cows in this experimental herd are split into two genetic merit groups, randomly assigned to high or low forage feed groups. The pedigree included 11,598 individuals over 27 generations, of which 71 sires and 478 dams had offspring with TL records. Leukocyte TLB was measured from whole blood samples collected through venepuncture within the first 14 days after birth. DNA was extracted from the blood samples and TLB was measured by qPCR as described before (Seeker et al. 2016). The raw TLB measures were log-transformed to ensure normal distribution.

Sources of variance in TLB

The sources of variance in TLB were examined by mixed linear models with the direct additive effect of the animal, and either maternal genetic or maternal permanent environment effects fitted as random. Their significance was tested individually through log-likelihood ratio test (LRT). Based on preliminary analyses including 21 fixed effects and covariates, fixed effects fitted were qPCR row and plate, binary lameness status of the calf's dam in lactation preceding the calf's birth, genetic group of the calf and the feed group of the calf's dam.

TLB and longevity

Longevity measures were herd life (HL – days between birth and cull date), productive life (PL – days between first calving and cull date) and binary survival to 12, 24, 36, 48, 60 and 72 months of age. The association between TLB and longevity was tested in three ways: 1) fitting TLB as a covariate in models fitted to longevity traits, 2) fitting a bivariate phenotypic analysis with longevity traits corrected for pertaining fixed effects and, 3) fitting a bivariate animal model with both traits corrected for the fixed effects. For binary survival traits, generalised linear mixed models logit link function were used. Significance of the residual and genetic correlations for HL and PL was tested with a LRT. Significance of phenotypic genetic and residual correlations between TLB and binary traits was tested using two-tailed t-test. Significance of TLB as a covariate was established from conditional Wald F statistic. Significance threshold was corrected for multiple testing using a Holm-Bonferroni test, assuming four independent hypotheses.

Genome-wide association study

For 246 animals Illumina Bovine SNP50 BeadChip array genotypes were available. Multidimensional scaling analysis confirmed the clustering of the individuals into the two known genetic groups. SNPs exceeding the minor allele frequency of 0.05, with p-value of Hardy-Weinberg Equilibrium test being $\leq 10^{-6}$ and proportion of individuals and markers with missing genotypes<0.05 were removed. After quality control 38,522 SNPs remained for further analysis. GEMMA software was used to run the GWAS based on a mixed model that included the genomic relationship matrix among individuals as a random effect, and

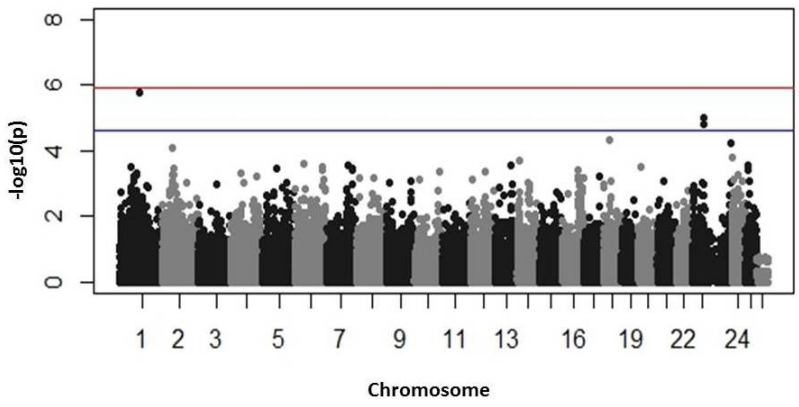
year/season of birth, genetic group and qPCR row as fixed effects. A genome-wide significance threshold was set at p=0.05 and a suggestive significance threshold of one false discovery per genome scan was applied. Bonferroni correction was used to account for multiple testing. Furthermore, using Ensembl, significant SNPs were mapped on the reference genome (Bos_taurus_UMD3.1) and a search for annotated genes within 0.5 MB windows around the significant SNP was performed. A pathway analysis was also performed using the IPA software.

Results and Discussion

TLB was moderately heritable (0.36±0.09). In the studied dataset maternal variances were not found to be significant, although a subset of the data indicated a significant maternal environment effect (0.33±0.10) (Ilska et al. 2017). The inconsistency of the estimates for maternal effects is common in studies of TL and exceeds the expectation of the heterogeneity of results caused by random error alone (Eisenberg 2014). Possible explanations include genuine differences between populations or an epigenetic inheritance of telomere sequences from particular germ cells. The significant effect of the lameness status of the dam on her offspring's TLB (offspring of lame cows in the preceding lactation had shorter TLB than those whose dams were not lame) supports the findings of associations between intra-uterine stress, hormone exposure and TL in offspring. Lameness is a major cause of chronic stress in cattle, and has been shown to affect the hormonal cascade, particularly cortisol and oestradiol levels.

The GWAS revealed three suggestive SNPs, on chromosomes 1 and 23 (Figure 1). The two significant markers on chromosome 23 were located within NEDD9, a gene involved in cell cycle progression and cytoskeletal regulation and has been previously associated with TL in humans. IPA analysis revealed the presence of significant pathways related with positive regulation of DNA metabolic process and response to hormones, positive regulation of cell cycle process, positive regulation to leukocyte chemotaxis, stress, immune response, cytokine signalling and cell proliferation.

Figure 1. Manhattan plot for TLB; x-axis is chromosome number; y-axis is -log10(p value); horizontal lines show the genome-wide (red) and suggestive genome-wide (blue) significance thresholds.



Associations with longevity

TLB showed significant associations with several measures of longevity (Table 1). The phenotypic correlation between TLB and survival was positive across all age groups and significant for ages 12, 24 and 36 months. Due to the relatively low number of records, the analyses for ages 48 and older failed to converge; thus more research is needed to examine these associations. The results of analyses of lifespan were significant for HL but not PL. A single significant estimate of genetic correlation at 0.76 (±0.23) was found between TLB and ST_M48 (heritability of 0.15±0.06), suggesting that animals with genetic propensity for longer TLB have a higher probability to survive to four years of age. A significant negative residual correlation was also estimated, which could potentially explain the relatively low estimates of the phenotypic correlations. In the literature, associations of TL with longevity are inconclusive and depend on the population studied, as well as on the period of longevity to be predicted. The associations between longevity and TL in dairy cows are likely to be different to those detected in humans and wild animals, as the longevity of a dairy cow does not represent her biological limits but depends on productivity, disease resistance and fertility.

Table 1.Regression coefficient (β) of TLB fitted as a covariate on longevity traits; correlation estimates from bivariate analyses of TL and longevity, from fixed effect and mixed animal model analyses. Standard errors in brackets. ST_M12 to ST_M48 – survival to age in months, HL – herd life. R_A, R_E and R_P – genetic, residual and phenotypic correlations respectively.

Longevity trait	β	Fixed effect model R_P	Mixed animal model		
			R_A	**R_E**	**R_P**
ST_M12	**2.55 (0.85) ***	**0.09 (0.02) ***	0.52 (0.30)	-0.04 (0.04)	0.11 (0.07)
ST_M24	2.14 (0.75)	**0.10 (0.02) ***	0.87 (0.44)	**-0.08 (0.04) ***	0.12 (0.06)
ST_M36	-1.18 (1.28)	**0.07 (0.02) ***	-0.06 (0.26)	0.08 (0.04)	0.05 (0.04)
ST_M48	1.01 (0.99)	*0.21 (0.02) ¥*	**0.76 (0.23) ***	**-0.21 (0.07) ***	0.13 (0.06)
HL	531.4 (224.3)	**0.12 (0.05) ***	-0.40 (0.24)	0.15 (0.09)	0.01 (0.06)

* Estimate significant after Holm-Bonferroni correction

¥ Analysis failed to converge.

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